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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 39/39, 39/00 — A1

(11) International Publication Number: WO 91/01143

(43) International Publication Date: 7 February 1991 (07.02.91)

(21) International Application Number: PCT/US90/03982

(22) International Filing Date: 16 July 1990 (16.07.90)

(30) Priority data:
379,742 14 July 1989 (14.07.89) US

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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: STABLE VACCINE COMPOSITIONS CONTAINING INTERLEUKINS

(57) Abstract

This invention pertains to interleukins-containing vaccine compositions, comprising a mixture of antigen and an adjuvant amount of an interleukin adsorbed onto a mineral in suspension and a preservative. Preferably, the mineral is alum. The interleukin can modulate the protective immune response to an antigen, while the mineral stabilizes the biological activity of the interleukin.

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STABLE VACCINE COMPOSITIONS CONTAINING INTERLEUKINS

Background

It is often desirable to enhance the immunogenic potency of an antigen in order to obtain a stronger immune response in the organism being immunized and to strengthen host resistance to the antigen-bearing agent. A substance that enhances the immunogenicity of an antigen with which it is injected is called an adjuvant. One of the more effective adjuvants is Freund's adjuvant, a water-in-oil emulsion. Freund's adjuvant is most effective when live or killed mycobacteria are suspended in the emulsion (Freund's complete adjuvant) along with antigen. However, the intense, chronic inflammation that results around deposits of the emulsion precludes the use of the adjuvant in man. Emulsions lacking mycobacteria (incomplete Freund's adjuvant) are less irritating and have been used in man. Another type of adjuvant is a suspension of minerals on which antigen is adsorbed.

Certain lymphokines have been shown to have adjuvant activity thereby enhancing the immune response to an antigen. For example, Good et al. demonstrate the use of recombinant human IL-2 (rhIL-2) adsorbed on alum to enhance the immune response to a malaria related antigen. This composition was prepared and used immediately and stability over time was not ascertained. Good, M.F. et al., J. Immunol. 141:972-977 (1988). Nakamura et al. demonstrated that interferon-gamma induced a two- to five-fold enhancement of antibody formation to several antigens. Nakamura et al., Nature

307: 381-382 (1984). Interleukins have also been shown to enhance an immune response to other antigens. Nencioni et al., J. Immunol. 139:800-804 (1987); Howard et al., EP285441.

Summary of the Invention

This invention pertains to stable interleukin-containing vaccine compositions comprising a mixture of antigen and an adjuvant amount of an interleukin adsorbed onto a mineral in suspension. The mixture can comprise a preservative. Interleukins, such as interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6 and interleukin-7 can be used as adjuvants in combination with antigen (particularly glycoconjugates) adsorbed onto a mineral suspension such as alum (e.g., aluminum hydroxide or phosphate) to modulate the immune response to the antigen. The vaccine compositions can be stored.

Detailed Description of the Invention

The vaccine compositions of this invention comprise an adjuvant amount of an interleukin in combination with the antigen adsorbed onto a mineral in suspension and a preservative. Preferably, the mineral is alum (e.g., aluminum hydroxide or aluminum phosphate) which is suspended in an aqueous medium.

The interleukin functions to modulate the immune response to the antigen, while the alum stabilizes the biological activity of the interleukin. In the absence of alum, interleukins have short half lives. Thus, the vaccine compositions of the present invention can be

stored for periods of time which would otherwise result in the destabilization of the interleukin. Stabilization will greatly extend the permissible time for the manufacture, shipment and storage of the vaccine formulations prior to administering of the vaccine formulation.

Several different interleukins can be used. These include interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, or mixtures of these. Portions of interleukins having immunomodulating activity can also be used. The preferred interleukin is interleukin-2.

Interleukin can be obtained from any suitable source. They can be produced by recombinant DNA methodology. For example, the genes encoding several human interleukins have been cloned and expressed in a variety of host systems, permitting the production of large quantities of pure human interleukin. Further, certain T lymphocyte lines produce high levels of interleukin thus providing a source of the interleukin.

The preservative can be any pharmaceutically acceptable preservative. These include thimerosal, phenol, m-cresol, benzyl alcohol, methyl or ethyl paraben, and 2-phenoxyethanol.

Interleukin can be used as adjuvant for many different types of antigens. In general, the antigens can be particulate antigens such as bacteria, viruses and macrocomponents of cells and soluble antigens such as proteins, peptides, glycoproteins and carbohydrates. Antigens of particular interest are viral or bacterial antigens, allergens, auto-immunity related antigens,

tumor-associated antigens, oncogene products, parasite antigens, fungal antigens or fragments of these. The antigens can be obtained from natural sources or they can be produced by recombinant DNA technology or other artificial means.

Among the bacterial antigens of interest are those associated with the human bacterial pathogens including, for example, typable and nontypable Haemophilus influenzae, Escherichia coli, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus pyogenes, Branhamella catarrhalis, Vibrio cholerae, Corynebacteria diphtheriae, Neisseria gonorrhoeae, Bordetella pertussis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, and Clostridium tetani. Some specific bacterial antigens include bacterial surface and outer membrane proteins (e.g. from Haemophilus influenzae, Neisseria meningitidis, Neisseria gonorrhoeae or Branhamella catarrhalis) and bacterial surface proteins (e.g. the M protein from Streptococcus pyogenes).

Viral antigens from pathogenic viruses include but are not limited to, human immunodeficiency virus (types I and II), human T-cell leukemia virus (types I, II and III), respiratory syncytial virus, hepatitis A, hepatitis B, hepatitis C, non-A and non-B hepatitis virus, herpes simplex virus (types I and II), cytomegalovirus, influenza virus, parainfluenza virus, poliovirus, rotavirus, coronavirus, rubella virus, measles virus, varicella, Epstein Barr virus, adenovirus, papilloma virus and yellow fever virus.

Several specific viral antigens of these pathogenic viruses include the F protein (especially antigens containing the F peptide 283-315 described in WO89/02935 entitled "Respiratory Syncytial Virus: Vaccines and Diagnostic Assays" by Paradiso, P. et al.) and the N and G proteins of respiratory syncytial virus (RSV), VP4 (previously known as VP3), VP6 and VP7 polypeptides of rotavirus, envelope glycoproteins of human immunodeficiency virus, the S and pre-S antigens of hepatitis B and herpes glycoproteins B and D.

Also of interest are various antigens associated with auto-immune diseases, such as rheumatoid arthritis and lupus erythematosus.

Of particular interest for use in a vaccine are capsular polymers (CP) produced by bacterial pathogens. Capsular polymers are sugar containing polymers, such as polymers of sugars, sugar acids, amino sugars, polyhydric alcohols and sugar phosphates. Several capsular polymers and oligomers are useful as vaccines.

The capsular polymers (CP) can be derived from many different types of bacteria. These types include Haemophilus influenzae, Streptococcus species including pneumoniae (particularly serotypes 1, 4, 5, 6A, 6B, 9V, 14, 18C, 19F, and 23F) pyogenes and agalactiae, Neisseria meningitidis (such as serogroup a, b and c), Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus.

Non-bacterial polymers can be derived from yeast and fungi, for example, Cryptococcus neoformans, or carbohydrate containing units found uniquely on cancer cells or those found associated with allergens.

The antigens of this invention can be used to elicit an immune response to an antigen in a vertebrate (such as a mammalian host). The method comprises administering to the animal, an immunologically effective dose of a vaccine composition comprising a mixture of an antigen and an adjuvant amount of an interleukin adsorbed onto a mineral in suspension and an added preservative. The vaccine compositions are useful for the prevention of microbial infections. The antigens may be administered in a pharmaceutically acceptable vehicle, such as physiological saline, or ethanol polyols (such as glycerol or propylene glycol). The vaccine composition may optionally comprise other adjuvants, such as vegetable oils or emulsions thereof, surface active substances, e.g., hexadecylamine, octadecyl amino acid esters, octadecylamine, lysolecithin, dimethyl-dioctadecylammonium bromide, N,N-dicoctadecyl-N'-N'bis (2-hydroxyethyl-propane diamine), methoxy-hexadecylglycerol, and pluronic polyols; polyamines, e.g., pyran, dextransulfate, poly IC, carbopol; peptides, e.g., muramyl dipeptide, dimethylglycine, tuftsin; immune stimulating complexes (ISCOMS); oil emulsions; and mineral gels. The antigens of this invention may also be incorporated into liposomes or ISCOMS. Supplementary active ingredients may also be employed.

The vaccines can be administered to a human or animal in a variety of ways. These include intradermal, transdermal (such as by the use of slow release polymers), intramuscular, intraperitoneal, intravenous,

subcutaneous, oral and intranasal routes of administration. The amount of antigen employed in such vaccine will vary depending upon the identity of the antigen employed. Adjustment and manipulation of established dosage ranges used with traditional carrier antigens for adaptation to the present vaccines is well within the ability of those skilled in the art. The vaccines of the present invention are intended for use in the treatment of both immature and adult warm-blooded animals, and in particular humans. Also, the use of the present composition is not limited to prophylactic application; therapeutic application are also contemplated (e.g., AIDS prophylaxis and therapy).

The adjuvant action of interleukin has a number of important implications: the adjuvant action of the interleukin can increase the concentration of protective antibodies produced against the antigen in the vaccinated organism. As a result, effective (i.e., protective) vaccination can be achieved with a smaller quantity of antigen than would be normally required. This reduction in the required amount of antigen may lead to more widespread use of vaccines which are difficult or costly to prepare. This is especially true in the developing nations which have very limited health care budgets and which face epidemics of respiratory diseases, diarrheal diseases and malaria. It may also provide for safer vaccination when the antigen is toxic at the concentration normally required for effective immunization. By reducing the amount of antigen, the risk of toxic reaction is reduced.

Interleukins, by means of their immunomodulating activity, can help evoke a protective immune response against marginally or non-immunogenic antigens. In this manner, vaccine compositions containing fragments of larger proteins, synthetic antigens or products of recombinant DNA technology may be made more potent by mixing them with interleukins.

Typically, vaccination regimens call for the administration of antigen over a period of weeks or months in order to stimulate a "protective" immune response. A protective immune response, is an immune response sufficient to protect the immunized host or subject from productive infection by a particular pathogen or pathogens to which the vaccine is directed. Interleukin, when coadministered with antigen and adsorbed onto a mineral in suspension can accelerate the generation of a protective immune response. This may reduce the time course of effective vaccination regimens. In some instances, it may result in the generation of a protective immune response in a single dosage. Further, vaccine formulations of this invention are sufficiently stable at 4°C to allow the manufacture, shipment and storage of the vaccine formulations.

This invention is further illustrated by the following examples.

EXAMPLES

Example I: Adjuvant Effect of rhIL-1 or rhIL-2 in the Presence of Alum With an HbOC Antigen Vaccine

A low dose (0.1 μg/mouse) dose of Haemophilus type b CRM conjugate (HbOC) was formulated with or without

alum (100 μg/mouse) and with various concentrations of rhIL-2, rhIL-1α or rhIL-1β ranging from 1 x 10^2 - 5 x 10^5 units/mouse. All vaccines were prepared on the day of immunization and maintained at 4°C until injected. Interleukins used in the preparation of the vaccines were either reconstituted on the day of formulation or were recovered from diluted stock (25 μg/ml) maintained at -70°C. Groups of Swiss-Webster mice (Taconic Farms, Germantown, NY) were injected intramuscularly (I.M.) at weeks 0 and 2 with 0.1 ml of the vaccine formulations. Serum samples were collected as indicated in the various Tables.

A. rhIL-2 as Adjuvant

Table 1 shows the results observed when rhIL-2 was administered as adjuvant in a mouse vaccination, both in the absence of alum [columns denoted (-)], and in combination with alum [columns denoted (+)]. Antibody concentrations are expressed as μg/ml and were determined at weeks 2, 4, and 6, following the administration of 0.1 μg of the antigen. The antigen used in these studies was HbOC. Doses of rhIL-2 administered ranged from 1 X 10^3 - 1 X 10^5 units/mouse.

TABLE 1

HbOC μg	Units rhIL	Week 2 (+)	Week 2 (-)	Week 4 (+)	Week 4 (-)	Week 6 (+)	Week 6 (-)
Series 1:							
0.1	10^3	4.70	0.28	7.89	3.46	8.16	3.62
0.1	10^4	2.25	0.51	9.39	5.01	17.77	5.83
0.1	10^5	0.37	0.55	4.71	3.04	5.31	2.91
0.1	--	0.46	<0.10	3.53	0.42	5.46	3.53
Series 2:							
0.1	10^2	1.02	0.40	6.32	4.21	5.90	3.32
0.1	10^3	0.54	0.75	5.77	4.73	5.60	4.61
0.1	10^4	1.68	<0.10	12.99	1.95	19.06	1.25
0.1	10^5	0.25	<0.10	5.94	5.46	3.91	3.82
0.1	--	0.53	0.30	7.09	2.64	8.30	2.82

As can be seen in the columns denoted (-) above, rhIL-2 without alum does have a stimulatory effect on antibody production For example, in two separate experiments, the antibody concentration present in the control samples was 0.42 μg/ml and 2.62 μg/ml.

The results observed when alum was included with rhIL-2 in the adjuvant mixture are shown in the columns denoted (+) in Table 1. Again, a significant stimulatory effect is seen. However, when alum is present, the magnitude of the antibody response is significantly increased over the response observed with otherwise identical samples without alum (vaccinations).

Again considering the 4 week time point, it can be seen that the with (+) alum antibody concentrations are

up to more than 10-fold greater than the corresponding without (-) alum antibody concentrations. Furthermore, there is an apparent rhIL-2 concentration dependence which was absent in the samples without (-) alum. Specifically, the correlation observed was that antibody concentration increased, as the rhIL-2 concentration decreased below 1×10^5. In the case of rhIL-2 with alum, the dose which appeared to stimulate the highest antibody production was approximately 1×10^4 units per mouse.

B. rhIL-1 as Adjuvant

Tables 2 and 3 show results obtained from immunizations in which rhIL-1 α and rhIL-1β were used as adjuvant, respectively. Table 2 presents data obtained from anti-PRP antibody determinations (expressed in μg/ml) at biweekly intervals post-immunization. Again, the experiments were conducted either with, (+), or without, (-), alum.

TABLE 2

HbOC μg	rhIL-1α (Units)	Week 2 (+)	Week 2 (-)	Week 4 (+)	Week 4 (-)	Week 6 (+)	Week 6 (-)
Series 1:							
0.1	10^3	1.96	1.02	14.63	4.94	13.25	4.93
0.1	10^4	0.70	2.54	7.16	5.11	12.44	11.17
0.1	10^5	0.33	1.49	4.69	7.02	6.14	8.73
0.1	-IL Control	0.46	<0.10	3.53	0.42	5.46	3.53
Series 2:							
0.1	10^2	<0.10	1.02	3.68	4.36	4.07	3.00
0.1	10^3	1.06	1.17	12.31	5.54	8.74	3.08
0.1	10^4	0.47	1.47	4.73	8.97	5.98	6.55
0.1	10^5	0.34	1.00	8.55	16.74	7.77	16.29
0.1	-IL Control	0.53	0.30	7.09	2.64	8.30	2.82

The results observed from rhIL-1α and rhIL-1β adjuvant mixtures were similar, overall, to those observed in the rhIL-2 series. Table 2, for example, shows that when rhIL-1α is administered as adjuvant without alum there is a stimulatory effect when compared with an otherwise identical vaccine without the lymphokine. As shown in the columns denoted (-), a tendency toward decreasing antibody production was observed as the amount of rhIL-1α in the adjuvant mixture was decreased from 1×10^5 to 1×10^2 units per mouse.

In the presence of alum, rhIL-1α also had a stimulating effect. Surprisingly, rhIL-1α demonstrated

increasing ability to stimulate the immune response as its concentration was decreased. The optimal adjuvant amount of rhIL-1α, in the presence of alum, was approximately 1×10^3. At such concentrations the antibody concentration was found to be 2-3 fold greater than the otherwise identical samples without (-) alum.

Similar results were observed when rhIL-1β was used as adjuvant, as shown in Table 3. The data in Table 3 summarizes the results of antibody concentration determinations made at weeks 2, 4, and 6, for varying concentrations of rhIL-1β as adjuvant, either with or without alum. In all but 6 of 21 experimental groups, the vaccine with (+) alum resulted in a higher antibody concentration than the vaccine without (-) alum.

TABLE 3

HbOC μg	rhIL-1β	Week 2 (+)	Week 2 (-)	Week 4 (+)	Week 4 (-)	Week 6 (+)	Week 6 (-)
Series 1:							
0.1	10^3	0.41	0.96	5.27	4.09	8.32	5.22
0.1	10^4	0.90	0.62	4.13	3.39	8.15	6.39
0.1	10^5	1.46	0.46	13.35	1.77	8.60	1.06
0.1	-IL Control	0.46	<0.10	3.53	0.42	5.46	3.53
Series 2:							
0.1	10^2	0.85	0.16	8.76	0.04	6.66	1.91
0.1	10^3	0.30	0.40	4.57	0.68	6.87	10.33
0.1	10^4	1.08	0.36	5.75	5.15	5.17	4.47
0.1	10^5	0.19	0.36	2.34	3.0	4.98	3.92
0.1	-IL Control	0.53	0.30	7.09	2.64	8.30	2.82

Example II: Adjuvant Effect of rhIL-1α, rhIL-1β, rhIL-2, and Mixtures Thereof on an RSV F Protein Vaccine

To determine whether interleukins can be used to enhance the antibody response to a protein vaccine, various concentrations of F protein of respiratory syncytial virus (RSV) were formulated with alum (final 100 μg/mouse) and with or without rhIL-2 or rhIL-1α at 1 x 10^4 or 1 x 10^5 units/mouse. Groups of Swiss-Webster mice (5 animals per group) were immunized intramuscularly at weeks 0 and 2. Animals were bled as indicated in Table 4.

Three doses of RSV F protein (1, 0.1 and 0.01 μg/mouse) were administered. Of these only 0.01 μg was suboptimal under the conditions employed. Comparison of the response seen in those groups receiving vaccines containing various interleukins with the control group receiving 0.01 μg of protein in alum alone revealed no significant effect (4 fold difference in titer) of rhIL-2 or rhIL-1α on the antibody response to F protein. However, the responses in the rhIL-2 treated groups were higher than controls at doses of 1×10^4 units/mouse which is similar to the results obtained in the HbOC studies. With IL-1α, both doses seem to show some improvement in antibody responses over controls. Interestingly, mixtures of 1×10^4 units/mouse of rhIL-2 and rhIL-1α did not show any indication of synergy but rather showed a slight decrease in the response relative to controls suggesting a possible antagonistic interaction of the interleukins.

TABLE 4

Adjuvant Effect of rhIL-2 on RSV F protein vaccine

μgF protein	rhIL	Units	Week 2	Week 4	Week 6
0.1	-IL				
	Control	--	141,657	2,341,756	2,454,512
0.1	IL-2	10^4	191,935	2,045,327	2,101,951
0.1	IL-2	10^5	309,797	2,275,311	2,311,326
0.01	-IL				
	Control	--	68,627	802,611	687,334
0.01	IL-2	10^4	145,467	1,580,552	1,699,135
0.01	IL-2	10^5	60,134	722,396	815,351
0.1	-IL				
	Control	--	141,657	2,341,756	2,454,512
0.1	IL-1α	10^4	123,446	1,861,917	1,771,952
0.1	IL-1α	10^5	79,386	1,185,475	1,214,008
0.01	-IL				
	Control	--	68,627	802,611	687,334
0.01	IL-1α	10^4	54,081	1,003,110	1,094,459
0.01	IL-1α	10^5	59,566	708,009	1,178,056
0.1	-IL				
	Control	--	141,657	2,341,756	2,451,512
0.1	Mix	10^4	207,410	2,593,957	2,353,912
0.01	-IL				
	Control	--	68,627	802,611	687,334
0.01	Mix	10^4	15,947	367,224	532,050
1.0	--	--	211,662	2,269,615	2,899,079

Example III. Single Dose Vaccination

Table 1 shows the results of an experiment demonstrating the immune response to HbOC with rhIL-2 with and without alum. At a concentration of 1 x 10^3 units rhIL-2, in the presence of alum, an HbOC based vaccine stimulated an antibody response of 4.7 μg/ml, after a single administration. Such an antibody concentration is above the threshold level generally accepted as necessary for protection.

Example IV. Adjuvant Stability

A. In Vitro Assay of rhIL-2 Stability

To study the stability of rhIL-2 in an alum containing composition, HbOC antigen (2.5 μg/mouse) was mixed with 10^4 units of rhIL-2 and adsorbed onto aluminum phosphate and stored at 4°C. Table 5 presents results observed in an in vitro stability assay. In the assay, 5 X 10^3 CTLL-2 cells were cultured with various concentrations of rhIL-2 standard and HbOC vaccines. Cells were incubated in RPMI media containing 10% FBS at 37° for 24 hours and pulsed with 1.0 μCi/well [^{3}H]-thymidine for 16 hours. To estimate the amount of rhIL-2 adsorbed to alum, the preparation was centrifuged to pellet the alum and the supernatent was assayed for rhIL-2 activity. It was determined that approximately 2/3 of the rhIL-2 is adsorbed onto alum. As shown in Table 5, when tested for rhIL-2 activity at time points following aluminum phosphate absorption, it was determined that the lymphokine maintained its activity for up to two weeks.

TABLE 5

In vitro assay of rhIL-2 stability (Incorporation of [3H]-thymidine at 1.0 U/ml IL-2 A ±CPM ± SD).

	Week 0	Week 1	Week 2
Baseline:			
Media (x cpm)	303 ± 41	1,540 ± 67	135 ± 49
Interleukin-2:			
BM product	16,179 ± 491	10,056 ± 689	13,323 ± 1428
Cetus product	28,300 ± 2250	25,681 ± 135	25,992 ± 868
HbOC vaccines:			
rhIL-2	24,188 ± 783	31,989 ± 2252	36,312 ± 3102
Alum/ rhIL-2	19,419 ± 1330	28,785 ± 1691	35,091 ± 690
Alum/ rhIL-2 supt	4,460 ± 205	5,716 ± 211	13,460 ± 1231
rhIL-2+50μg Alum	ND	ND	35,627 ± 2503

B. In Vivo Assay of rhIL-2 Stability

An in vivo assay of rhIL-2 stability was designed. Mice were immunized with an HbOC/Alum/IL-2 vaccine which had been stored as described above. Four groups of two DBA/2 mice were immunized with 10μg (protein) HbOC in CFA, Alum, rhIL-2/Alum, or rhIL-2 on 3 consecutive weeks. Lymph nodes were removed one week after injection and single cell

suspension was obtained. 3×10^5 LNC were cultured with mitogens and various concentrations of DT, CRM, and TT. Cells were incubated in RPMI media containing 1.0% NMS at 37°C for 3 days, pulsed with 1.0 μCi/well [3H]-thymidine for 16 hrs, and harvested for counting on LS counter. A significant T cell response was observed in the HbOC-CFA group. In weeks 2 and 3, however, rhIL-2 induced an augmented T cell response. Furthermore, even HbOC alone appears to protect rhIL-2 from degradation.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims:

TABLE 6

In Vivo Stability of IL-2 with HbOC Vaccine

Maximum Incorporated [3H]-thymidine as SI ± SD.

	Week 1 HbOC Priming In:			
In Vitro Challenge:	CFA	Alum*	IL-2/Alum*	IL-2*
Media (x cpm)	454 ± 22	190 ±19	386 ±35	125 ± 8
CA 1.0μg/ml	43.3± 3.9	89.7± 3.9	58.4± 5.1	136.9± 2.4
LPS 50μg/ml	129.4± 8.7	189.9± 7.7	69.0± 4.5	103.9± 6.6
DT	17.5± 1.2	4.4± 0.04	1.4± 0.3	1.8± 0.1
CRM	162.0± 14.9	9.8± 0.3	0.8± 0.1	0.8± 0.1
TT	0.8± 0.07	0.4± 0.03	0.1± 0.0	0.6± 0.05
	Week 2			
Media	1,185 ±175	590 ±42	552 ±48	187 ±11
CA 1.0μg/ml	40.6± 3.4	57.5± 1.8	53.1± 0.9	165.1±11.5
LPS 50μg/ml	71.5± 1.2	89.9± 3.7	142.1± 8.7	203.5±17.8
DT	16.5± 0.4	1.1± 0.0	1.3± 0.2	2.9± 0.0
CRM	50.6± 0.5	1.3± 0.0	10.5± 0.3	1.6± 0.0
TT	0.6± 0.1	0.1± 0.0	0.1± 0.0	0.4± 0.0
	Week 3			
Media	519 ± 59	416 ± 9	442 ±63	966 ±51
CA 1.0μg/ml	45.9± 0.3	41.0± 2.8	46.4± 0.9	27.5± 2.1
LPS 50μg/ml	129.2± 7.6	119.4± 5.3	126.5± 6.3	81.9± 3.1
DT	5.6± 0.8	NA	2.2± 0.2	NA
CRM	98.1± 8.1	17.9± 1.4	25.2± 1.6	31.4± 2.6
TT	0.4± 0.1	0.2± 0.0	0.51 0.0	0.1± 0.0

*These groups received 2.0% NMS instead of 1.0%

CLAIMS

1. A stable vaccine composition, comprising a mixture of an antigen and an adjuvant amount of an interleukin adsorbed onto a mineral in suspension and a pharmaceutically acceptable preservative.

2. The vaccine composition of Claim 1, wherein the interleukin is selected from the group consisting of interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, or mixtures thereof.

3. The vaccine composition of Claim 2, wherein the interleukin is human interleukin-2.

4. The vaccine composition of Claim 1, wherein the mineral suspension is an aqueous suspension of alum.

5. The vaccine composition of Claim 1, wherein the antigen is an antigen selected from the group consisting of bacteria, viruses, macro-components of cells, proteins, peptides, glycoproteins, carbohydrates, parasites, fungi, oncogene products and cancer cells.

6. A vaccine composition of Claim 5, wherein the bacterial antigen is from a bacterial pathogen.

7. The vaccine composition of Claim 6, wherein the bacterial pathogen is selected from the group consisting of Haemophilus influenzae, Escherichia coli, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus pyogenes, Branhamella catarrhalis, Vibrio cholerae, Corynebacteria diphtheriae, Neisseria gonorrhoeae, Bordetella pertussis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae and Clostridium tetani.

8. The vaccine composition of Claim 1, wherein the antigen is a bacterial capsular polymer, oligmer, or fragment thereof.

9. The vaccine composition of Claim 8, wherein the bacterial capsular polymer, oligomer or fragment thereof is Haemophilus influenzae, Streptococcus pheumoniae, Neisseria meningitidis, Klebsiella pneumoniae, Pseudomonas aeruginosa or Staphylococcus aureus.

10. The vaccine composition of Claim 1, wherein the antigen is coupled to a glycoconjugate.

11. The vaccine composition of Claim 10, wherein the glycoconjugate comprises a bacterial toxin of diphtheria, tetanus, pertussis or CRM, or toxoid thereof.

12. The vaccine composition of Claim 10, wherein the glycoconjugate comprises polyribosylribitol-phosphate and CRM_{197} of diphtheria toxin.

13. A vaccine composition of Claim 1, wherein the bacterial antigen is a bacterial surface or outer membrane protein.

14. The vaccine composition of Claim 13, wherein the bacterial surface or outer membrane protein is of Haemophilus influenzae, Neisseria meningitidis, Neisseria gonorrhoea or Branhamella catarrhalis.

15. A vaccine composition of Claim 13, wherein the bacterial surface protein is the M protein from Streptococcus pyogenes.

16. A vaccine composition of Claim 5, wherein the viral antigen is selected from the group consisting of F protein of respiratory syncytial virus, N protein of respiratory syncytial virus, G protein of respiratory syncytial virus, VP4 polypeptide of rotavirus, VP6 polypeptide of rotavirus, VP7 polypeptide of rotavirus, envelope glycoproteins of human immunodeficiency virus, herpes glycoproteins B and D and the S and pre-S antigens of hepatitis B.

17. A vaccine composition of Claim 1 wherein the preservative is thimerosal, phenol, benzyl alcohol, methyl or ethyl paraben, 2-phenoxyethanol or m-cresol.

18. A stable vaccine composition comprising, a mixture of an antigen and an adjuvant amount of interleuken-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6 or interleukin-7, adsorbed onto a mineral in suspension.

19. A vaccine composition of Claim 18, further comprising a preservative.

20. A vaccine composition of Claim 19, wherein the preservative is thimerosal, phenol, m-cresol, benzyl alcohol, methyl or ethyl paraben, or 2-phenoxyethanol.

21. The vaccine composition of Claim 18, wherein the mineral suspension is an aqueous suspension of alum.

22. The vaccine composition of Claim 18, wherein the antigen is an antigen selected from the group consisting of bacteria, viruses, macro-components of cells, proteins, peptides, glycoproteins, carbohydrates, parasites, fungi, oncogene products and cancer cells.

23. A vaccine composition of Claim 22, wherein the bacterial antigen is from a bacterial pathogen.

24. The vaccine composition of Claim 23, wherein the bacterial pathogen is selected from the group consisting of Haemophilus influenzae, Escherichia coli, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus pyogenes, Branhamella catarrhalis, Vibrio cholerae, Corynebacteria diphtheriae, Neisseria gonorrhoeae, Bordetella pertussis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae and Clostridium tetani.

25. The vaccine composition of Claim 18, wherein the antigen is a bacterial capsular polymer, oligmer or fragment thereof.

26. The vaccine composition of Claim 25, wherein the bacterial capsular polymer, oligomer or fragment thereof is Haemophilus influenzae, Streptococcus pheumoniae, Neisseria meningitidis, Klebsiella pneumoniae, Pseudomonas aeruginosa or Staphylococcus aureus.

27. The vaccine composition of Claim 18, wherein the antigen is coupled to a glycoconjugate.

28. The vaccine composition of Claim 27, wherein the glycoconjugate comprises a bacterial toxin of diphtheria, tetanus or pertussis, or CRM or toxoid thereof.

29. The vaccine composition of Claim 28, wherein the glycoconjugate comprises polyribosylribitol-phosphate and CRM_{197} of diphtheria toxin.

30. A vaccine composition of Claim 18, wherein the bacterial antigen is a bacterial surface or outer membrane protein.

31. The vaccine composition of Claim 30, wherein the bacterial surface or outer membrane protein is of Haemophilus influenzae, Neisseria meningitidis, Neisseria gonorrhoeae or Branhamella catarrhalis.

32. A vaccine composition of Claim 30, wherein the bacterial surface protein is the M protein from Streptococcus pyogenes.

33. A vaccine composition of Claim 22, wherein the viral antigen is selected from the group consisting of F protein of respiratory syncytial virus, N protein of respiratory syncytial virus, G protein of respiratory syncytial virus, VP4 polypeptide of rotavirus, VP6 polypeptide of rotavirus, VP7 polypeptide of rotavirus, envelope glycoproteins of human immunodeficiency virus, herpes glycoprotein B and D and the S and pre-S antigens of hepatitis B.

34. A method of eliciting an immune response against an antigen, comprising administering to a vertebrate host an effective amount of a stable vaccine composition, comprising a mixture of an antigen and an adjuvant amount of an interleukin adsorbed onto a mineral in suspension and a pharmaceutically acceptable preservative, in a pharmaceutically acceptable vehicle and optional adjuvant.

35. The method of Claim 34, wherein the interleukin is selected from the group consisting of interleukin-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin 7 or mixtures thereof.

36. The method of Claim 34, wherein the mineral suspension is an aqueous suspension of alum.

37. The method of Claim 34, wherein the antigen is an antigen selected from the group consisting of bacteria, viruses, macro-components of cells, proteins, peptides, glycoproteins, carbohydrates, parasites, fungi, oncogene products and cancer cells.

38. A method of eliciting an immune response against an antigen, comprising administering to a vertebrate host an effective amount of a stable vaccine composition comprising, a mixture of an antigen and an adjuvant amount of interleuken-1α, interleukin-1β, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6 or interleukin-7 adsorbed onto a mineral in suspension, in a pharmaceutically acceptable vehicle and optional adjuvant.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/03982

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/39, A 61 K 39/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	The Journal of Immunology, volume 141, no. 3, 1 August 1988, The American Association of Immunologists, (Baltimore, US), M.F. Good et al.: "Recombinant human IL-2 overcomes genetic nonresponsiveness to malaria sporozoite peptides. Correlation of effects with biologic activity of IL-2", pages 972-977 see the abstract; Material and Methods (cited in the application)	18,21-33
Y		1-17,19,20
	--	
X	The Journal of Immunology, volume 140, no. 1, 1 January 1988, The American Association of Immunologists, (Baltimore, US) A. Weinberg et al.: "Recombinant interleukin 2 as an adjuvant for vaccine-induced protection. Immunization of guinea pigs with herpes simplex virus subunit vaccines", pages 294-299	18,21-33

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2nd November 1990	10. 12. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	miss T. MORTENSEN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	see the abstract; Material and Methods	
Y		1-17,19,20
	--	
Y	The Journal of Immunology, volume 139, no. 3, 1 August 1987, The American Association of Immunologists, (Baltimore, US), L. Nencioni et al.: "In vivo immunostimulating activity of the 163-171 peptide of human IL-1beta", pages 800-804 see the abstract (cited in the application)	1-17,19,20
	--	
P,X	EP, A, 0351876 (AJINOMOTO CO. INC.) 24 January 1990 see example 4	18,21-33
Y		1-17,19,20
	--	
P,X	EP, A, 0343480 (BIOTEST PHARMA GmbH) 29 November 1989 see the whole document	18,21-33
Y		1-17,19,20
	--	
Y	Bundesverband der Pharmazeutischen Industrie e.V., "Rote Liste 1989", 1989, Editio Cantor, (Aulendorf/Würtemberg, DE) see abstract no. 74060	1-17,19,20
	--	
P,A	GB, A, 2217600 (NATIONAL INSTITUTE OF HEALTH) 1 November 1989 see the whole document	10-12,27-29

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers *, because they relate to subject matter not required to be searched by this Authority, namely:

* 34-38
See PCT Rule 39.1(iv): methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods

2. [ ] Claim numbers....., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9003982 SA 38895

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/11/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0351876	24-01-90	JP-A- 2036129	06-02-90
EP-A- 0343480	29-11-89	DE-A- 3818054	30-11-89
		JP-A- 2111727	24-04-90
GB-A- 2217600	01-11-89	DE-A- 3911442	02-11-89
		FR-A- 2629717	13-10-89

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82